THE ACTION OF CERTAIN DRUGS ON THE INSECT CENTRAL NERVOUS SYSTEM

K. D. ROEDER

Tufts College, Massachusetts

The purpose of this paper is to describe the effects of certain drugs on general reflex activity in insects, and to compare the pharmacology and physiology of the insect and vertebrate nervous systems. The literature reveals few positive observations of the effect of injected drugs on insects, though in general it seems that insects are less responsive than other invertebrates.

Material and Methods

The material for these experiments consisted of mature female praying mantids (*Mantis religiosa*) and mature cockroaches (*Periplaneta americana*). The various drugs used were made up in .6 per cent saline shortly before injection. The drugs were introduced by injection into the head capsule with a small syringe and fine hypodermic needle. Since the insect circulation is rather sluggish, there is only slight general distribution of an injected solution, and the site of injection determines to some extent the effect of a drug. An injection of .01 cc. into the head capsule appears to reach the supraesophageal and to some extent the subesophageal ganglion, but has little or no effect on the rest of the nerve cord. Records were kept in the form of written observations and 16 mm. moving pictures. Little attempt will be made to present quantitative results, since it is difficult to inject an exactly measured dose into an unanaesthetised insect, and there is no satisfactory way of quantifying the resultant change in behavior without giving false impressions.

Experimental

Strychnine

The normal posture and degree of activity of the mantis are variable, and have been described at length (Roeder, 1937). Injection of .02 mg. strychnine causes a slight drop in tonus and decrease in antennal vibration. This effect is very transitory, lasting only a few minutes. Larger doses of from .05 to .1 mg. result in a pronounced departure from the normal within 3 to 5 minutes. First the antennal vibrations become slower and the movements of the mouth-parts very sluggish. With large doses movements of the head appendages cease entirely. The tonus of the trunk muscles drops, and the insect takes on a crouching posture owing to the sagging of the long prothorax. The degree of locomotor activity varies, though at first there is an increase, the insect walking slowly and restlessly. The movements of the legs are coördinated, and identical with normal locomotor movements. Owing to insensitivity of the head appendages and the crouching posture, the mantis is unable to climb up or over any obstacle, and since it moves in a straight line, it eventually becomes wedged in a corner or under some object. Mechanical stimulation of the antennae or photic stimulation of the eyes fails to produce any response, and the whole head appears to be quite insensitive. These symptoms: a drop in tonus, increased locomotor activity, and paralysis of the head appendages also follow destruction of the cerebral ganglia, therefore the effect of strychnine on the mantis central nervous system is entirely depressant. Experiments with cockroaches yielded essentially similar results. The insects became very quiet, and there was little mouth-part or antennal movement. Both mantids and cockroaches recovered completely from the effects of strychnine within an hour.

The effect of strychnine on these insects is then in direct contrast to its well-known effect on the vertebrate spinal cord, where it supposedly lowers thresholds between neurons in the cord, and is capable of turning an inhibitory process into an excitatory one. Many invertebrates show a comparable reversal of inhibition (Knowlton and Moore, 1917; Moore, 1918; Crozier and Federighi, 1924; and Crozier, 1927, 1930), though strychnine is recorded as having little or no effect on insects (Crozier, 1922; Crozier and Pilz, 1924). Many other papers are summarised in a review by Poulsson (1920). The only vertebrate organ which is depressed by strychnine is the heart (Poulsson, 1920).

Pilocarpin

This drug belongs to a group which has a specific stimulating effect on parasympathetic effectors (Kuntz, 1934). In vertebrates it increases salivary secretion, produces vasodilation, has a vagus-like effect on the heart, and increases contractility in the stomach and intestine, while its action is blocked by atropin (Dixon and Ransom, 1924). No record of its effect on the vertebrate central nervous system could be discovered.

If pilocarpin is injected into the head capsule of a praying mantis, it produces a state of great excitation. The minimum dose producing observable effect is about .01 mg. while doses of from .02 to .1 mg. bring about a change in behavior within thirty seconds. The walking legs become flexed, the prothorax raised, and the head ventrally flexed.

apparently due to an increase in tonus of the ventral neck muscles. There is an increase in amplitude and rate of antennal movement, and the mandibles are opened and shut, producing a clicking sound. There are spasmodic movements of the raptorial legs, consisting of flexions and extensions, and occasionally the whole body is swayed rapidly from side to side. All reflex activity increases except locomotion. Only three out of thirty insects showed any tendency to walk, either spontaneously or on stimulation. Recovery occurs rapidly and the insect is normal within an hour.

High muscle tone and inhibition of locomotor activity are typical of an insect with the right and left cerebral ganglia separated. This is interpreted as being due to increased activity of the tonus and inhibitory locomotor centres when released from the inhibitory effect of the contralateral ganglion (Roeder, 1937). Since pilocarpin when applied to the cerebral ganglia of an intact insect has the same effect as median division, it must have an excitatory action upon these centres. This can be substantiated by removing the cerebral ganglia prior to injection. An insect so treated walks rapidly in a crouching position. Since the cerebral centres are now absent the pilocarpin exerts its excitatory effect upon the locomotor centre in the subesophageal ganglion with consequent activity.

Pilocarpin has a comparable effect on cockroaches. A solution of the drug was injected into the head in doses of approximately .01 cc. A solution of 1 : 500 produced immobility and apparent paralysis but solutions of 1 : 500 to 1 : 5,000 caused intense excitation. It is impossible to give an adequate description of the behavior because so many things occur simultaneously. The mouth-parts and antennae are in continual movement, the head is moved from side to side and ventrally flexed, and cleaning movements are made with the legs, which are often abnormally extended. Occasionally there are violent cramps and spasms involving the whole of the body musculature and very rapid side-to-side swaying movements similar to those observed in treated mantids. More dilute solutions of 1 : 5,000 to 1 : 25,000 either have no noticeable effect or produce only a restlessness and general increase in activity. The above symptoms appear within one minute of injection and last for about thirty minutes. Recovery is usually complete. The excitation produced by pilocarpin seems to indicate a similarity between the nervous systems of these two insects and the vertebrate parasympathetic. This similarity leads to an investigation of the response to acetylcholine and eserine.

Acetylcholine and Eserine

Recent work has shown that acetylcholine acts as mediator between parasympathetic nerves and autonomic effectors, and between pre- and postganglionic fibres in the autonomic ganglia. Naturally produced acetylcholine is destroyed very rapidly by the enzyme esterase, but it can be protected from destruction by a drug, eserine. By its protective action eserine renders autonomic effectors and postganglionic fibres more sensitive to introduced acetylcholine, and, by preventing the immediate destruction of naturally produced acetylcholine, can prolong the postganglionic response to a preganglionic stimulus (Cannon and Rosenblueth, 1937).

For these experiments cockroaches only were used, as mantids were not available. Acetylcholine hydrochloride was dissolved in .6 per cent saline, and injected into the head, in .01 cc. amounts. Strengths from 1 : 500 to 1 : 25,000 were used but in no case was any change in behavior noted. Since the acetylcholine was unprotected by eserine its effect may have been only transitory, and therefore would have escaped attention. In order to prolong the effect, if any, cockroaches were first injected with eserine. This drug had an unexpected effect, which was comparable with, but more pronounced than, that of pilocarpin. Eserine alone in dilutions of 1 : 250 to 1 : 500 produced an almost instantaneous spasm and immobility from which the insect did not recover, while solutions of 1 : 500 to 1 : 2,500 caused enormous reflex activity, which consisted of rapid antennal quivering, continuous mouth-part activity, movements of the head, great increase in extensor leg tonus and general spasms. Usually the insects did not walk, but one or two were so active that no observation could be made. Solutions more dilute than 1 : 5,000 either had no effect or produced only a slight restlessness.

It can be seen that this strong eserine effect made it impossible to test the effect of acetylcholine protected by eserine. Though the presence of acetylcholine in the nervous system of these insects has not been demonstrated, the action of eserine could be explained by its presence.

Atropin

The specific action of this drug is to block transmission of impulses in autonomic ganglia and to parasympathetic effectors. It also renders these structures insensitive to parasympatheomimetic substances such as acetylcholine and pilocarpin (Cannon and Rosenblueth, 1937). From the foregoing experiments it might be expected that atropinised insects would fail to respond to pilocarpin, and would also show a decreased reflex activity. The answer to the second question is somewhat doubtful. Atropinised cockroaches remain very inactive for long periods, though they are able to respond if disturbed. Cockroaches were first brought into a condition of great activity with .01 cc. of 1 : 500 pilocarpin, and then subjected to an injection of atropin. This failed to decrease the pilocarpin effect to a noticeable extent. If the insects were first injected with atropin and then given an injection of 1 : 500 pilocarpin, they showed either normal or decreased activity in eight out of ten cases. Two insects showed a very brief phase of excitation, lasting a few seconds, and then became inactive, but in no case was there the prolonged and intense reflex activity which occurs if such a dose of pilocarpin is administered alone. Therefore, previous treatment with atropin protects cockroaches against the pilocarpin effect, just as it does in the vertebrate parasympathetic.

Conclusions

The following conclusions seem justified:

(1) The effect of strychnine on the reflex activity of the mantis and cockroach indicates that the central nervous systems of these insects and vertebrates differ in certain respects.

(2) The action of pilocarpin, eserine, and atropin on the reflex activity of these insects indicates that there is a pharmacological similarity between their nervous systems and the vertebrate parasympathetic.

It must be recognized that the experiments outlined above are suggestive rather than conclusive, since the methods used are in many ways unsatisfactory. First, there is no way of knowing the exact concentration of drug actually reaching the cerebral ganglia, partly because it is not an easy matter to inject small exact amounts into a struggling insect, and partly because of the sluggish and casual nature of the insect circulation. Second, there is no way of telling exactly what structures are being reached by the injected drug. From information available on the functions and interrelations of the cerebral ganglia in the mantis (Roeder, 1937) it seems highly probable that these structures are being directly affected. The great activity of the mouth-parts indicates that the subesophageal ganglion is also affected to some extent, though it is hard to say to what extent the drugs are affecting sense organs and neuro-muscular junctions. Third, observation of changes in the general reflex activity is a complex and unsatisfactory criterion. However, a satisfactory technique avoiding some of these difficulties has been developed, and it is hoped that more extended conclusions can be submitted in a later paper.

The similarity of the insect central nervous system and the vertebrate parasympathetic immediately suggests the presence of a chemical substance such as acetylcholine, which would serve as mediator in the transfer of impulses from neuron to neuron in the central nervous system. No proof of the presence of such a substance is presented here, though the action of eserine is suggestive. Acetylcholine unprotected by eserine produces no noticeable effect when injected, but an insect is capable of such rapid and brief responses that one would expect very rapid destruction or removal of any substance mediating the transfer of impulses.

The question as to whether these findings apply generally to insects cannot be given an adequate answer. It has been reported that strychnine produces little or no excitation in caterpillars (Crozier and Pilz, 1924), while pilocarpin raises sensory thresholds in the same insects (Crozier, 1922), while crustacea (Clarke and Wolf, 1932; Viehoever and Cohen, 1937) and many invertebrates respond to strychnine in a vertebrate-like manner. It would seem that a generalization with respect to insects must await a further investigation.

Summary

1. Adult praying mantids and cockroaches were injected in the head with solutions of various drugs, and the changes in general reflex activity were recorded.

2. Strychnine caused a decrease in reflex activity in both insects, large doses causing complete cessation of antennal and mouth-part movement. This is contrary to its effect on the vertebrate spinal cord.

3. Pilocarpin and eserine bring about an increase in mouth-part and antennal movement, head movement and extensor leg tonus, and produce spasmodic twitching and general contraction of the body musculature. In the case of pilocarpin this effect is prevented by atropin.

4. Acetylcholine alone has no detectable effect on activity. Its effect, if any, when injected with eserine is masked by the excitatory effect of eserine.

5. It is concluded that, in their reactions to drugs, the nervous systems of the two insects studied show little similarity to the vertebrate central nervous system, but considerable similarity to the vertebrate parasympathetic.

LITERATURE CITED

Cannon, W. B., and A. Rosenblueth, 1937. Autonomic Neuro-effector Systems. The Macmillan Company, New York.

Clarke, G. L., and E. Wolf, 1932. The mechanisms of tropistic reactions in Daphnia and the strychnine effect in Daphnia. *Jour. Gen. Physiol.*, **16**: 99–105.

Crozier, W. J., 1922. "Reversal of inhibition" by atropine in caterpillars. *Biol. Bull.*, **43**: 239–245.

Crozier, W. J., 1927. Galvanotropism and "reversal of inhibition" by strychnine. *Jour. Gen. Physiol.*, **10**: 395–406.

Crozier, W. J., 1930. Reversal of galvanotropism in echinoderms. *Jour. Gen. Psychol.*, **3**: 268–276.

Crozier, W. J., and H. Federighi, 1924. Suppression of phototropic circus movements of Limax by strychnine. *Jour. Gen. Physiol.,* **7**: 221–224.

Crozier, W. J., and G. F. Pilz, 1924. Central nervous excitation by alkaloids in insects. *Am. Jour. Physiol.,* **69**: 41–43.

Dixon, W. E., and F. Ransom, 1924. Pilocarpin, physostigmin, Arecolin. Gifte welche bestimmte Nervenendigungen erregen. Handbuch der Experimentellen Pharmacologie 2 (2) 746–785. Herausgegeben von A. Heffter. Julius Springer, Berlin.

Knowlton, F. P., and A. R. Moore, 1917. Note on the reversal of reciprocal inhibition in the earthworm. *Am. Jour. Physiol.,* **44**: 490–491.

Kuntz, A., 1934. The Autonomic Nervous System. Lea and Febiger, Philadelphia.

Moore, A. R., 1918. Reversal of reaction by means of strychnine in planarians and starfish. *Jour. Gen. Physiol.,* **1**: 97–100.

Poulsson, H., 1920. Die Strychningruppe. Handbuch der Experimentellen Pharmacologie 2 (1). Herausgegeben von A. Heffter. Julius Springer, Berlin.

Roeder, K. D., 1937. The control of tonus and locomotor activity in the praying mantis (Mantis religiosa L.). *Jour. Exper. Zoöl.,* **76**: 353–374.

Viehoever, A., and I. Cohen, 1937. Mechanism of strychnine. *Am. Jour. Pharm.,* **109**: 285–316.